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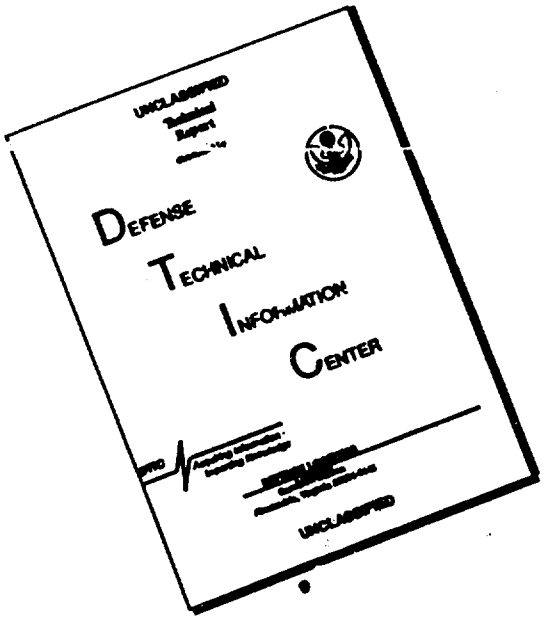

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Dissolved CO_2: determination in small sample volumes using the Scholander 0.5-ml analyzer

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D'AOUST, BRIAN G. *Dissolved CO_2: determination in small sample volumes using the Scholander 0.5-cc analyzer.* J. Appl. Physiol. 30(1): 142–145. 1971.—A simple procedure is described which utilizes an unmodified Scholander 0.5-ml analyzer for extraction and analysis of total dissolved CO_2 in small sample volumes and allows recovery of the analyzed CO_2 for determination of radioactivity. This permits measurement of specific activity on one sample. The method is portable, rapid and useful over a concentration range of 10^4.

$^{14}CO_2$; *CO_2 determination; CO_2 specific activity; CO_2 microanalysis;* gasometric analysis; field methods

MICROMETHODS for determining specific activity of dissolved CO_2 (1, 3–5, 9), bicarbonate, or carbonate are limited chiefly by the convenience and sensitivity of the total CO_2 determination, whereas 10^{-5} μc of ^{14}C is easily measured by liquid scintillation counting. I describe here a simple procedure using an unmodified Scholander 0.5-ml gas analyzer (7) for determining total dissolved CO_2 in small volumes of fluid. All of the sample may then be recovered for counting of radioactivity, thus allowing specific activity determinations on the same sample. As little as 0.020 ($\pm$0.004) μmoles and as much as 5.00 ($\pm$0.05) μmoles may be analyzed in samples as small as accurately measurable up to 100 μl. Thus a range in concentration of 10^4 can be analyzed and specific activities as low as 10^{-3} mc/mmole determined on 0.020 μmoles of CO_2.

PRINCIPLE

The method involves extracting the dissolved CO_2 from a known volume of acid solution into a known volume of CO_2-free air. The CO_2 is then reabsorbed, the volume change recorded, and aliquots of the trapped CO_2 contained in a known volume of solution can be counted in an ion chamber or liquid scintillation counter, the latter providing the most sensitivity. Since both liquid and gas phase volumes are accurately known, empirical corrections for extracted CO_2 may be determined for any particular set of conditions.

PROCEDURE

Familiarity with the apparatus, as detailed in the original paper (7) is assumed in the following procedure, which uses the original reagents. For CO_2 in strongly alkaline samples, as in CO_2 traps, different strength reagents must be used. This was found practical, subject to the requirements of having the water-vapor tension of acid and alkali exactly matched and the need for more time to dissipate the larger amount of heat of neutralization following addition of the alkali. The original reagents (7) are well suited to analysis of physiological strength solutions and natural waters and are used in the method as it is described here. All precautions described in the original paper are critical in the present application for maximum sensitivity.

Figure 1 shows the analyzer (labeled as in the original report) with the sample being introduced into the reaction chamber (b) with a 10-μl Hamilton syringe. The life of the Piccolyte coating is only slightly decreased by this procedure. A length of PE-10 catheter tubing can be used to extend the needle tip, if the amount of sample available permits; however, the sample should not be stored exposed to the tubing as considerable CO_2 can be lost.

1) To prepare for sample introduction, the chamber b is rinsed with acid rinsing solution by raising and lowering I several times with S-2 in position III.

2) The mercury level is then accurately adjusted to the top of the capillary in a using the micrometer with S-2 in position I.

3) The micrometer is then adjusted to read zero (S-2 in position II).

4) With S-2 in position I, 200 micrometer divisions (four turns of the micrometer screw) of fresh acid rinse are drawn into the capillary and chamber b.

5) The acid remaining is removed from a with suction or a syringe and a small amount of CO_2 absorber is delivered with a hypodermic syringe into the depression around the top of the capillary protruding into a.

6) Stopcock S-1, fitted with a 5.0-ml disposable glass syringe barrell filled with ascarite or baralyme, is then placed open in the top of a.

7) The top of the acid is then adjusted to the capillary mark, and the micrometer readjusted to zero.

8) The chamber is then shaken to absorb any CO_2 in a into the small volume of alkali placed there in *step 5*, and 2,000 micrometer divisions (40 turns of the micrometer screw) of CO_2-free air are drawn evenly into chamber b, ejected back through chamber a, and then drawn back into b again. This assures CO_2-free air in chamber b. (There are alternative ways to insure that CO_2-free air is drawn into chamber b. The procedure used here was developed in order to leave the apparatus free for conventional gas analysis.)

9) S-1 is then gently removed from a and a small drop of acid placed inside the capillary with a hypodermic syringe and a no. 21 (or smaller) needle.

10) The alkali is removed with suction from a and the latter is rinsed twice with the acid rinse to assure that no CO_2 previously trapped in alkali enters chamber b.

11) Chamber a is next filled to several millimeters above the top of the capillary with acid which is then drawn with the micrometer screw down to the piccolyte coating (7).

12) The sample is now delivered as shown in Fig. 1 on to the top of the mercury in b. During sample delivery the acid in a is maintained just at the junction of chamber b and the capillary by adjusting the micrometer. After delivery, the syringe is removed, the acid level slowly and evenly pushed up almost to chamber a, and excess acid removed leaving in the capillary a reference drop which is now adjusted to the mark. Care should

be taken during this step to prevent acid from flowing past the coating into chamber b, particularly during the next step when introducing the sample. The total volume of solution will be made up of the initial volume of acid, the sample volume, and the alkali. If excess acid is added accidentally during sample addition the correction factor B will differ from that determined from Fig. 2, *A* and *B*.

13) S-1 is now replaced on a, closed, and the analyzer is shaken for 2.0 min to assure maximum CO_2 extraction, during which time the reference drop is maintained approximately at the mark with the micrometer screw.

14) After extraction the reference drop is gently lowered to the coating to pick up any drops of acid shaken free during extraction and then readjusted to the mark. This is repeated until the micrometer reading is constant. This reading is then accepted as V_1. A small hand lens is necessary for reading the micrometer with maximum sensitivity. This reading most critically determines the accuracy of the analysis. A dirty capillary will prevent reliable analysis of small quantities of CO_2. The reader is referred to discussion of these factors in the original paper (7).

15) The analyzer is next tilted gently to admit from c into b a volume of CO_2 absorber approximating that of the extracted solution.

16) The analyzer is then shaken for 30 sec, again maintaining the reference drop at the mark using the micrometer screw.

17) Finally, the reference drop is lowered to the coating, slowly raised back to the mark and V_2 read from the micrometer.

When it is desired to recover $^{14}CO_2$ for determination of radioactivity, it is necessary to measure the total volume of solution in chamber a. This is done as follows. The gas phase is ejected (S-2 in position 1), and the gas/liquid miniscus is set to the mark.

FIG. 1. Scholander 0.5-ml gas analyzer, showing introduction of sample with Hamilton syringe placed through acid (400 ml water, 1.00 ml concd H_2SO_4, 72 g Na_2SO_4, 21.0 ml glycerol) in the thermobarometer chamber a, into CO_2-free air-filled chamber b. Black arrow shows tip of needle; white arrow shows 5.0-ml glass syringe barrel filled with baralyme and attached to S-1 for CO_2 absorption during the filling of b; c denotes CO_2 absorber (100 ml H_2O, 11 g KOH, 40 mg $K_2Cr_2O_7$); I is the leveling bulb which is connected to the analyzer through S-2. Stopcock S-2 positions shown at lower left. Spring-steel wire clips, fashioned so as to continually apply pressure to the rubber stoppers on each side of the glass, are shown in position. These improve accuracy by reducing elasticity. Further details should be sought in ref. 7.

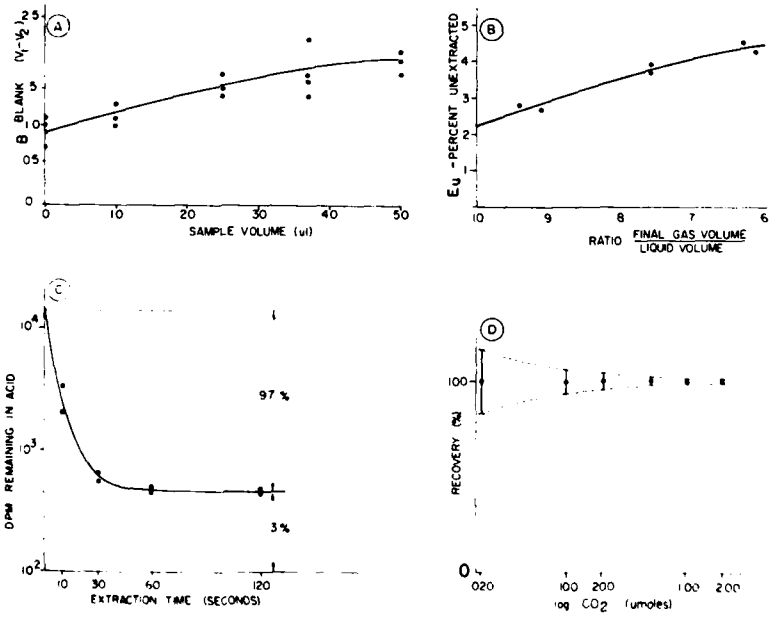

FIG. 2. *A:* changes in micrometer readings $V_1 - V_2$ (*ordinate*) "B" associated with increasing sample size (*abscissa*) when carried out with CO_2-free samples. This reflects water vapor tension changes caused by diluting the acid with sample. The analysis is improved to the extent that this correction is minimized. *B:* percentage of CO_2 unextracted E_u (*ordinate*) plotted against different gas liquid ratios (*abscissa*) corresponding to a sample volume range of 1.0–50.0 µl, determined by extracting known amounts of $^{14}CO_2$ for 2.0 min, ejecting the gas phase and counting the radioactivity remaining in the acid. *C:* time course of extraction of CO_2 in 20-µl sample from acid carried out as described above (*ordinate:* total counts remaining, *abscissa:* time). *D:* total CO_2 in samples analyzed expressed as percent (*ordinate*) plotted against $\log_{10}$ of total CO_2 in sample (*abscissa*). Each value calculated with equation shown in text using corrections B and E_u taken from graphs *A* and *B*. Dotted line shows decrease in standard deviation with increasing total amount of CO_2 analyzed.

TABLE 1. *Total CO_2 in whole blood*

Van Slyke		Scholander 0.5 ml	
Blood vol, ml	Total CO_2, mEq/liter	Blood vol, µl	Total CO_2, mEq/liter
1.00	21.8	5.8	21
1.00	22.0	10.8	20
		10.8	19
		10.8	22
		10.8	23
	$\bar{X}$ = 21.9		$\bar{X}$ = 20.1
			±SD = ±1.8

With S-2 in position II, the micrometer screw is set exactly at 1,000.0 or another high reading (V_a), and with S-2 in position I the solution is driven up into a (which must first be dried with suction) until the liquid/mercury junction is at the mark. The micrometer is then read, giving V_b. Aliquots of the solution in a may now be taken for determination of radioactivity, and the specific activity of the $^{14}CO_2$ determined.

All determinations of radioactivity in this technique were done by evolving the trapped CO_2 from acid into 0.20 ml of hydroxide of Hyamine in a polyethylene diffusion cup (Konte's Glass Co., Vineland, N.J.) and counting the entire cup in a toluene-methanol 15:2 cocktail with a Beckman LS 250 counter.

CALCULATIONS

The total amount of CO_2 in the sample is calculated as follows:

$$\mu\text{moles total } CO_2 \text{ (STP)} = \frac{(V_1 - V_2 - B) \times F}{(100 - E_u)} \times \frac{273}{273 + {}^\circ C}$$

where V_1 and V_2 are the initial and final readings of the micrometer before and after CO_2 absorption, B is a correction, empirically determined, which compensates for differences in the vapor tension of the acid/sample solution and that of the alkali according to the volume of sample analyzed (Fig. 2*A*), F is a conversion factor (which is constant for any one apparatus and temperature) whose units are (µmoles)·(micrometer div^{-1}) at STP, and E_u expresses the fraction of CO_2 unextracted at equilibrium (Fig. 2*B*). The value F is determined by measuring the volume, in micrometer divisions, of an accurately weighed quantity of mercury (near 0.500 g) delivered into b with a Hamilton syringe. Thus

$$F_{STP} = \frac{\text{wt of Hg delivered}}{22.26 \cdot (\text{micrometer div}) \cdot (\text{density Hg at T }{}^\circ C)} \cdot \frac{\text{mg}}{\mu l \cdot \mu\text{mole}^{-1}(\text{mg}/\mu l)}$$

A value of 0.0167 was determined in the present analyzer. Corrected for a temperature of 23 C, F decreased to 0.0154 µmoles/micrometer division.

The liquid volume in microliters is determined as $(V_a - V_b) \cdot L$, where V_a and V_b are the initial and final readings, respectively, of the micrometer during ejection of the liquid phase, and L is the volume calibration factor of the system (in units of $mm^3 \cdot div^{-1}$ wt Hg/(density Hg × micrometer divisions). Knowing the volume of the liquid prior to absorption of CO_2, the gas/liquid volume ratio $V_L/(V_a - V_b)$ is calculated and the percent of CO_2 unextracted, E_u, read off the ordinate in Fig. 2*B*. This curve is determined using the above procedure to extract, from different sample volumes, a known amount of radioactive $^{14}CO_2$, rapidly ejecting the gas phase and determining the residual radioactivity after a 2.0-min extraction (Fig. 2*C*). The maximum correction E_u found with a 100.0-µl sample of 0.0458 M $NaHCO_3$ was 5.8%. This correction should be determined on solutions approximating the ionic strength and alkalinity of the sample to be analyzed. The distribution law holds that with temperature, and liquid and gas volumes held constant the mass ratio of gas in the liquid and gas phases is independent of pressure. However, the CO_2 solubility of the liquid phase will change with the volume and composition of the sample. The range of values of E_u shown in Fig. 2*B* covers a sample volume range of 1–100 µl of samples whose alkalinity reaches 50 mEq liter.

Figure 2*D* shows the mean and standard deviation, expressed as percent, of 10 analyses each of increasing amounts of CO_2 in samples ranging in concentration from 0.4 to 45.8 mM. In each case the values have been corrected with values of B and E_u taken from Fig. 2, *A* and *B*, respectively. Included in the 10 analyses of each amount of CO_2 on the abscissa of Fig. 2*D* are samples of different volumes and concentrations. For example when total CO_2 was 0.02 µmoles, sample volumes used included 0.5 µl of 0.040 M $NaHCO_3$ and 50.0 µl of 0.0004 M $NaHCO_3$.

The method has been used in studies of CO_2 equilibration in the swim bladder of juvenile sunfish, *Lepomis megalotis* (unpublished data). An additional advantage of the method is that small volumes of whole blood can be directly analyzed as described above, suggesting considerably wider applications. Table 1 compares several analyses of one sample of human heparinized whole blood done with the Van Slyke and the Scholander 0.5-ml analyzer.

Reproducibility is lowered by blood, chiefly due to small amounts of protein sticking to the capillary and changing the distribution of forces on the reference drop. This is largely eliminated by thorough rinsing between analyses. The blood is well broken up during extraction, and proteins denatured during the acid extraction appear to become more soluble and less adhesive on addition of the alkali.

Obvious advantages of the method, namely, the portability of the apparatus, the small sample size required, the range of concentrations which may be analyzed with no need for modifications (2, 8, 10), make it a useful field method. For example, 20 µl of seawater have enough CO_2 to analyze at the lower limit of sensitivity (approximately 0.040 µmoles). This will allow reliable measurements of metabolic $^{14}CO_2$ to be made with small aquatic organisms in small volumes of seawater or larger volumes of freshwater (6).

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13. ABSTRACT

A simple procedure is described which utilizes an unmodified Scholander 0.5-ml analyzer for extraction and analysis of total dissolved carbon dioxide in small sample volumes and allows recovery of the analyzed carbon dioxide for determination of radioactivity. This permits measurement of specific activity on one sample. The method is portable, rapid and useful over a concentration range of 10000.

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Field methods						